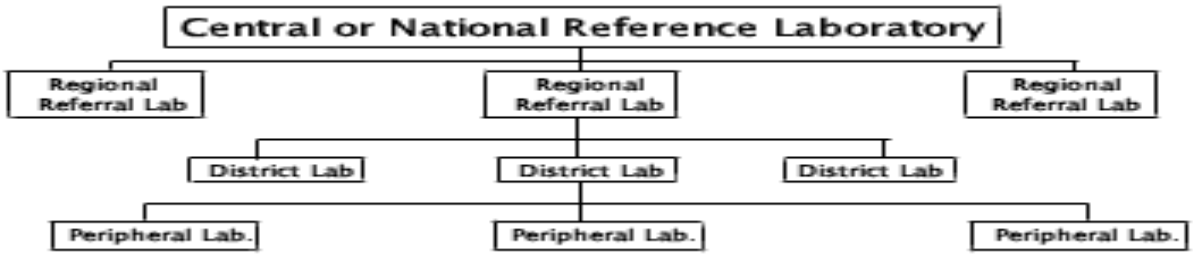
Medical Labs

The organization of laboratories in any country is usually a three or four tier system with various possible functional linkages between them. One possible way of networking of laboratories is shown in Figure.

Figure: Networking of Laboratories



Peripheral laboratory services

Peripheral laboratories are located at the point of first contact of patients with the health care services. In most developing countries these are available only at primary health center or community health center (upgraded primary health center) level.

The functions performed by these laboratories:
These laboratories provide technical support for preventive, curative and promotive services for the individual as well as the community.

Staff

The staff in peripheral laboratories should include one technician and one laboratory assistant/attendant.

Equipment and supplies

Necessary equipment and supplies include good microscopes, centrifuges, autoclaves, refrigerators, balances, pH meters, incubators, water bath, transport media, glassware, sterile swabs, and reagents for staining (eg. Gram, Albert, Ziehl Neelsen, Romanowsky), reagents for chemical examination of urine, kits and reagents for rapid diagnostic tests, sterilized syringes and needles, micropipettes and tips as well as sterile collection bottles for blood/serum and water analysis.

Tests to be performed

Peripheral laboratories are expected to undertake tests of public health as well as clinical relevance. Among the tests of public health relevance, diseases of greater epidemiological importance should be accorded priority. Testing of environment samples (especially water) also falls into the priorities of public health relevance. Certain rapid serological tests may be of use in studying epidemiological patterns of important diseases and the same can also be performed at peripheral laboratories.

The tests to be performed by peripheral laboratories are subject to the availability of resources, manpower, technology and prevalence of various diseases in the area catered to by the laboratory. A suggested list is provided in Table.

Table: Suggested tests to be performed at peripheral laboratories

Procedure/Specimen	For detection/diagnosis of
Urine examination	Pus cells, RBCs Albumin Sugar
Stool examination	Ova and cysts
Stained smears Throat specimen Sputum CSF (pyogenic and tubercular) Peripheral blood smear	 Diphtheria Tuberculosis Meningitis Malaria, filariasis
Rapid diagnostic tests	HIV Hepatitis B surface Ag Syphilis Meningococcal disease

Intermediate laboratory services

In most developing countries, intermediate laboratories are located at district or the regional headquarters and may act as clinical as well as public health laboratories.

The functions performed by these laboratories:

1. Laboratory support to clinical diagnosis/public health Quality assurance Logistic and technical support, Training of staff for peripheral laboratories
2. Supervision and monitoring of peripheral laboratories

Intermediate laboratories help in the diagnosis and treatment of the individual patient and are also used as public health laboratories for epidemiological surveillance and control of diseases in the community. These laboratories also serve as links between peripheral laboratories and the state/central laboratory for the following:

- Collection, storage and analysis of data.
- Distribution of reagents, media, laboratory manuals.
- Purchase of equipment.
- Supervision of peripheral laboratories.
- To conduct external quality assessment scheme (EQAS) for peripheral laboratories.
- To take part in EQAS organized by the state/central laboratories.
- To send samples to higher/reference laboratories for characterization of isolate/confirmation of diagnosis.

Staff

Qualified pathologist/ microbiologist
(Doctor of Medicine/diploma in clinical pathology)

Technicians

DMLT (diploma in medical laboratory technology) with experience
Laboratory Assistants (DMLT)
Laboratory attendants
Cleaner
Clerk-cum-storekeeper

Since it may not be possible to have a full-time epidemiologist, at least part time help of an epidemiologist should be available.

Equipment

Binocular microscope	2	Colorimeter	1
Dark-field microscope	1	Refrigerator	1
Inoculating chamber	2	Balances	2
Centrifuge	2	pH meter	1
Autoclave	2	Inspissator	1
Incubator	2	Distil water apparatus	1
Hot air oven	1	Micropipettes	as per workload
Water bath	2	Tips for pipettes	as per workload
VDRL shaker	1		

The laboratory diagnosis of an infectious disease begins with the collection of a clinical specimen for examination or processing in the laboratory (the right one, collected at the right time, transported in the right way to the right laboratory). Proper collection of an appropriate clinical specimen is the first step in obtaining an accurate laboratory diagnosis of an infectious disease. Guidelines for the collection and transportation of specimens should be made available to clinicians in a lucidly written format.

The guidelines must emphasize two important aspects:

- Collection of the specimen before the administration of antimicrobial agents.
- Prevention of contamination of the specimen with externally present organisms or normal flora of the body.

Collection and transportation of specimens

- Apply strict aseptic techniques throughout the procedure.
- Wash hands before and after the collection.
- Collect the specimen at the appropriate phase of disease.
- Make certain that the specimen is representative of the infectious process (e.g. sputum is the specimen for pneumonia and not saliva) and is adequate in quantity for the desired tests to be performed.
- Collect or place the specimen aseptically in a sterile and/or appropriate container.
- Ensure that the outside of the specimen container is clean and uncontaminated.

- Close the container tightly so that its contents do not leak during transportation.
- Label and date the container appropriately and complete the requisition form.
- Arrange for immediate transportation of the specimen to the laboratory.

Criteria for rejection of specimens

Criteria should be developed by a laboratory on the basis of which the processing of a specimen may not be done by the laboratory. The following are some examples:

- Missing or inadequate identification.
- Insufficient quantity.
- Specimen collected in an inappropriate container.
- Contamination suspected.
- Inappropriate transport or storage.
- Unknown time delay.
- Haemolysed blood sample.

Collection of specimens

The clinical state of the patient will not necessarily be reflected by the result of laboratory investigation despite correct laboratory performance unless the specimen is in optimal condition required for the analysis. Some of the important specimens and their proper collection and transportation methods are described here so as to ensure quality.

Blood

Whole blood is required for bacteriological examination. Serum separated from blood is used for serological techniques. Skin antisepsis is extremely important at the time of collection of the sample. Tincture of iodine (1-2%), povidone iodine (10%) and chlorhexidine (0.5% in 70% alcohol) are ideal agents. However, some individuals may be hypersensitive to iodine present in some of these.

While collecting blood for culture, the following points must be remembered:

- Collect blood during the early stages of disease since the number of bacteria in blood is higher in the acute and early stages of disease.

- Collect blood during paroxysm of fever since the number of bacteria is higher at high temperatures in patients with fever.
- In the absence of antibiotic administration, 99% culture positivity can be seen with three blood cultures.
- Small children usually have higher number of bacteria in their blood as compared to adults and hence less quantity of blood needs to be collected from them (Table).

Table: Volume of blood to be collected at different ages

Age	Volume in 2 bottles
< 2 years	2 ml
2-5 years	8 ml
6-10 years	12 ml
>10 years	20 ml

Cerebrospinal fluid (CSF)

Examination of CSF is an essential step in the diagnosis of any patient with evidence of meningeal irritation or affected cerebrum. Almost 3-10 ml of CSF is collected and part of it is used for biochemical, immunological and microscopic examination and remaining for bacteriological or fungal examination. The following important precautions need to be taken for CSF collection and transportation:

- Collect CSF before antimicrobial therapy is started.
- Collect CSF in a screw – capped sterile container and not in an injection vial with cotton plug.
- Do not delay transport and laboratory investigations.
- Transport in a transport medium if delay in processing is unavoidable.
- CSF is a precious specimen, handle it carefully and economically. It may not be possible to get a repeat specimen.
- Perform physical inspection immediately after collection and indicate findings on laboratory requisition form.
- Store at 37^{o}C, if delay in processing is inevitable.

The characteristics of the appearance of CSF are outlined in Table.

Table: Appearance and interpretations of CSF

Clear and colourless	Normal
Clear with Tyndall effect *(sparkling appearance against incident light)*	High protein content
Clear yellowish	Old haemolysis
Clear red	Fresh haemolysis
Turbid blood-stained	Haemorrhage
Turbid white	High cell or protein content
Turbid clot *(after overnight storage)*	Fibrin clots

Sputum

Sputum is processed in the laboratory for etiological investigation of bacterial and fungal infections of the lower respiratory tract. It is of utmost importance in the diagnosis of pulmonary tuberculosis.

- Select a good wide-mouthed sputum container, which is preferably disposable, made of clear thin plastic, unbreakable and leak proof material.
- Give the patient a sputum container with the laboratory serial number written on it. Show the patient how to open and close the container and explain the importance of not rubbing off the number written on the side of the container.
- Instruct the patient to inhale deeply 2-3 times, cough up deeply from the chest and spit in the sputum container by bringing it closer to the mouth.
- Make sure the sputum sample is of good quality. A good sputum sample is thick, purulent and sufficient in amount (2-3 ml).

Give the patient an additional container with laboratory serial number written on it for an early morning specimen. Explain to the patient to rinse his/her mouth with plain water before bringing up the sputum.

Urine

Under normal circumstances urine is sterile. The lower part of the urethra and the genitalia are normally colonised by bacteria, many of which may also cause urinary tract infection. Since urine is a good growth medium for all sorts of bacteria, proper and aseptic collection assumes greater importance for this specimen.

For microbiological examination urine must be collected as a "clean catch-mid-stream" specimen.

Urine specimens should be transported to the laboratory within one hour for bacteriological examination, because of the continuous growth of bacteria *in vitro* thus altering the actual concentration of organisms.

Stool

Stool specimens for the etiological diagnosis of acute infectious diarrhoeas should be collected in the early stage of illness and prior to treatment with antimicrobials. A stool specimen rather than a rectal swab is preferred.

- The stool specimen should not be contaminated with urine.
- Do not collect the specimen from bed pan.
- Collect the specimen during the early phase of the disease and as far as possible before the administration of antimicrobial agents.
- 1 to 2 gm. quantity is sufficient.
- If possible, submit more than one specimen on different days.
- The fresh stool specimen must be received within 1-2 hours of passage.
- Store at 2-8^{o}C.
- Modified Cary and Blair medium is recommended as a good transport medium. It is a very stable medium and can be stored for use in screw – capped containers. It is a semi-solid transport medium. At least two swabs should be inoculated. Most pathogens will survive for up to 48 hours at room temperature. Specimens are unacceptable if the medium is held for more than one week or if there is detectable drying of the specimen.

Alternative transport media are Venkataraman-Ramakrishnan medium (V-R fluid) or alkaline peptone water. VR fluid should be prepared in 30 ml (1 oz) screw capped bottles (MacCartney bottles). It preserves vibrios for more than six weeks and has also proved to be a very convenient medium for transportation as it can be kept at room temperature after collection of the specimen.

Throat swab

- Depress the tongue with a tongue blade.
- Swab the inflammed area of the throat, pharynx or tonsils with a sterile swab taking care to collect the pus or piece of membrane.

- Transport in sterile transport tube.

Bone marrow

Bone marrow is collected by a doctor who is well trained in this procedure

- Decontaminate the skin overlying the site from where specimen is to be collected with 70% alcohol followed by 2% tincture of iodine.
- Aspirate 1 ml or more of bone marrow by sterile percutaneous aspiration.
- Collect in a sterile screw-cap tube.
- Send to laboratory immediately.

Rectal swab

- Insert swab at least 2.5 cm beyond the anal sphincter so that it enters the rectum.
- Rotate it once before withdrawing.
- Transport in Cary and Blair or other transport medium.

Transportation of specimens

Specimens to be sent to other laboratories require special attention for safe packing of the material. Guidelines are usually issued by national authorities and the same should be strictly followed. For hand-carried transportation over a short distance, the specimen should be placed upright in appropriate racks. For long distance transportation, it should be placed in three containers, i.e:

- A primary container which has the specimen and is leak proof with a screw-cap.
- A secondary container which is durable, waterproof and made of metal or plastic with a screw-cap. It should have enough absorptive material to absorb the contents of the primary container should the latter break or leak. On its outside, the details of the specimen should be pasted.
- A tertiary container is usually made of wood or card box. It should be capable of withstanding the shocks and trauma of transportation. Dry ice can be kept between this and the secondary container along with sufficient absorbents and provision for the escape of carbon dioxide to prevent a pressure build-up inside .

In general, most specimens should be processed in the laboratory within 1 to 2 hours after collection. In practice, a 2-to 4-hour time limit is probably more practical during a normal working day. The laboratory must be organized to permit processing of the specimens as soon as they arrive, and the collection of most specimens should be limited to the working hours of the laboratory. However, some arrangements must be made to allow for the initial handling of the few specimens that have to be collected outside of the laboratory's working hours.

Safety in Laboratories

Laboratory safety is a vital component of functioning of any laboratory. Safety procedures and precautions to be followed in the microbiology laboratory should be designed to:

- Restrict microorganisms present in specimens or cultures to the vessels in which they are contained.
- Prevent environmental microorganisms (normally present on hand, hair, clothing, laboratory benches or in the air) from entering specimens or cultures and interfering with the results of the studies.

Laboratory biosafety levels

Four biosafety levels have been recommended based on the infectiousness of the agent/s.

Biosafety Level -1 (BSL-1): Adherence to standard microbiological practices. No special requirement as regards containment equipment.

Biosafety Level-2 (BSL-2): In addition to the use of standard microbiological practice, laboratory coats, decontamination of infectious wastes, limited access, protective gloves and display of biohazard sign and partial containment equipment are the requirements for this level.

Most peripheral and intermediate laboratories need BSL-1 or BSL-2 laboratory facilities.

BSL-3: In addition to BSL-2, it has special laboratory clothing, controlled access to laboratory and partial containment equipment.

BSL-4: BSL-3 plus entrance through change room where laboratory clothing is put on, shower on exit, all wastes are decontaminated before exit from the facility. It requires maximum containment equipment.

- **Laboratory facilities in BSL-2**

- Laboratory should be designed in such a way that it can be easily cleaned.
- Laboratory contains a sink for washing.
- Laboratory tops are impervious to water but resistant to acids, alkalies and organic solvents.
- An autoclave to decontaminate infectious material is available.
- Illumination is adequate for all laboratory activities.
- Storage space is adequate.

Preventive measures against laboratory infections

These are aimed to protect workers, patients and cultures. Following steps are suggested:

- Perform adequate sterilization before washing or disposing waste.
- Provide receptacle for contaminated glassware.
- Provide safety hood.
- Ensure that tissues are handled and disposed of properly.
- Promote regular hand washing and cleaning of bench tops.
- Ensure use of gloves.
- Provide mechanical pipetting devices.
- Protect patients from laboratory personnel with skin or upper respiratory tract infections.
- Provide special disposal containers for needles and lancets.

Hypodermic syringes and needles

Accidents involving the use of syringes and needles while drawing blood from patients or performing experiments on laboratory animals are among the most common causes of occupational infections in laboratories and health care facilities. They account for almost 25% of the laboratory-acquired infections that occur by accidents. The practices which are recommended for hypodermic needle and syringes are:

- Avoid quick and unnecessary movements of the hand holding the syringe.
- Examine glass syringes for chips and cracks, and examine needles for barbs and plugs.
- Use needle locking syringes only and be sure that needle is locked securely.
- Wear surgical or other gloves.
- Fill syringes carefully to minimize air bubbles and frothing.
- Expel excess air, liquid and bubbles vertically into a cotton pledget moistened with suitable disinfectant.
- Do not use syringe to forcefully expel infectious fluid into an open vial for mixing. Mixing with a syringe is appropriate only if the tip of the needle is held below the surface of the fluid in the tube.
- Do not bend, shear, recap or remove the needle from syringe by hand.
- Place used needle-syringe units directly into a puncture-resistant container and decontaminate before disassembly, reuse or disposal.

Opening containers

The opening of vials, flasks, petri dishes, culture tubes, and other containers of potentially infectious materials poses potential but subtle risks of creating droplets, aerosols or contamination of the skin or the immediate work area. The most common opening activity in most health care laboratories is the removal of stoppers from containers of clinical materials. It is imperative that specimens should be received and opened only by personnel who are knowledgable about occupational infection risks. Various precautions that can be taken in this regard are:

- Open containers with clinical specimens in well-lighted and designated areas only.
- Wear a laboratory coat and suitable gloves.
- If possible, use a plastic-backed absorbent paper towel to:
  – facilitate clean-up
  – reduce generation of aerosols
- Specimens which are leaking or broken may be opened only in safety cabinets.

Tubes containing bacterial cultures should be handled with care. Vigorous shaking of liquid cultures creates a heavy aerosol. When a sealed ampoule containing a lyophilized or liquid culture is opened, an aerosol may be created. Ampoules should be opened in a safety cabinet.

Laboratory access

- As far as possible children and pregnant women visitors should not enter the microbiological laboratories.
- Appropriate signs should be located at points of access to laboratory areas directing all visitors to a receptionist or receiving office for access procedures.
- The universal biohazard symbol shall be displayed at specific laboratories in which manipulations of organisms with moderate and heavy risk are being carried out. Only authorized visitors shall enter the laboratory showing universal biohazard sign. Doors displaying biohazard symbol shall not be propped open, but shall remain closed when in use.

Clothing

- All employees and visitors in microbiological laboratories shall wear laboratory clothing and laboratory shoes or shoe covers.
- Disposable gloves shall be worn wherever radiological, chemical, carcinogenic materials or virus preparations of moderate to high risk are handled.
- Laboratory clothings including shoes shall not be worn outside the work area.

Accidents in laboratory

In the microbiological laboratory, bacterial infections pose the most frequent risk. The important diseases/organisms are:

Hepatitis B virus, Shigella spp., HIV , Salmonella spp. including S typhi, Brucella spp., Bacillus anthracis, Leptospires, Yersinia pestis, Mycobacteria spp., Histoplasma

Accidents and spills

The order of priorities is as follows:

- Protection of personnel
- Confinement of contamination
- Decontamination of personnel
- Decontamination of area involved

- Decontamination of skin.

The area is washed thoroughly with soap and water. Detergents or abrasive materials must not be used and care must be taken not to damage the skin.

- Decontamination of cuts\eyes.

These are irrigated with water taking care to prevent the spread of contamination from one area to another.

- Decontamination of clothing.

Contaminated garments should be removed immediately and placed in a container. They should not be removed from the spill location until contamination has been monitored.

- Decontamination of work surfaces

  - Flood the total spillage area including the broken container with disinfectant.
  - Leave undisturbed for 10 minutes.
  - Mop with cotton wool or absorbent paper.
  - Wear disposable gloves, apron and goggles.
  - If a dustpan and brush or forceps have been used these too require disinfection.
  - For blood or viruses, hypochlorites (10 gm/L) are used.
  - Do not use hypochorite solution in centrifuges.
  - Use activated gluteraldehyde (20 gm/L) on surfaces for viral decontamination.
  - Place all potentially contaminated materials in a separate container and retain until monitored.
  - Restrict the entry to such an area until contamination monitoring has been carried out.

Management of laboratory accidents

An adequately equipped first-aid box should be kept in the laboratory in a place that is known and accessible to all members of staff. The box must be clearly marked and preferably be made of metal or plastic to prevent from damage by pests. A medical officer should be consulted regarding the contents of the box. A

first-aid chart giving the immediate treatment of cuts, burns, poisoning, shock and collapse, should be prepared and displayed in the laboratory.

General laboratory directions for safety

The salient general laboratory directions which must be obeyed by all are:

- Long hair should be bound back neatly away from shoulders.
- Do not wear any jewellery to laboratory sessions.
- Keep fingers, pencils, bacteriological loops etc. out of your mouth.
- Do not smoke in the laboratory.
- Do not lick labels with tongue (use tap water).
- Do not drink from laboratory glasswares.
- Do not wander about the laboratory; uncontrolled activities cause:
  –accidents
  –distract others
  –promote contamination
- Do not place contaminated pipettes on the bench top.
- Do not discard contaminated cultures, glasswares, pipettes, tubes or slides in wastepaper basket or garbage can.
- Avoid dispersal of infectious materials.
- Operate centrifuges, homogenizer and shakers safely.
- Immunize the laboratory workers against vaccine-preventable diseases such as hepatitis B, meningococcal meningitis, rabies, etc.

Quality Assurance:

Quality Assurance (QA) is a wide ranging concept covering all matters that individually or collectively influence the quality of a product. It denotes a system for continuously improving reliability, efficiency and utilization of products and services. In the context of quality assurance two important definitions need to be clearly understood:

i. **Internal Quality Control (IQC):** which denotes a set of procedures undertaken by the staff of health facility (medical, paramedical workers as well as laboratorians) for continuously and concurrently assessing laboratory work so that quality results are produced by the laboratory for supporting quality health care at patient and community levels.

ii. **External Quality Assessment (EQA):** is a system of objectively assessing the laboratory performance by an outside agency. This assessment is retrospective and periodic but is aimed at improving the IQC.

IQC and EQA are complementary in ensuring the reliability of the procedures, results and quality of the product.

What is the objective of QA?

- **QA programmes are required for the following reasons:**

- To improve the quality of health care.
- To generate reliable, reproducible results.
- To establish inter-laboratory comparability in laboratory testing.
- To establish the credibility of the laboratory among doctors and the public at large.
- Motivating the staff for further improvement.
- Prevention of legal complications which may follow poor quality results.

Factors affecting the quality

It is commonly believed that the quality of laboratory results solely depends upon the laboratory undertaking this analysis. However, there are many pre-analytical and post-analytical factors which influence the quality of the end results to a very significant extent. The principle of "GIGO" – "Garbage in Gabage Out" very well applies to the laboratory tests also. Some of the important factors influencing quality are listed here:

i. **Specimen:** This is the single most important factor. Selection of the right sample, collection in a right manner, adequate quantity, proper transportation to the laboratory, and processing of the sample before testing, are crucial factors.
ii. **Personnel:** The quality of the laboratory results generated is directly proportional to the training, commitment and motivation of the technical staff.
iii. **Environmental factors:** Inadequate lighting, workspace or ventilation or un safe working conditions may influence the laboratory results.
iv. **Analytical factors:** The quality of reagents, chemicals, glassware, stains, culture media,use of standard procedures and reliable equipment all

influence laboratory results. Failure to examine a sufficient number of microscope fields can lead to false negative results.

v. **Post analytical factors:** Transcription errors, incomplete reports, and improper interpretation can adversely influence the laboratory results.

➢ IQC – the mainstay of QA

The backbone of a good quality assurance program is a good IQC. Intermediate and peripheral laboratories must put in place various IQC procedures and may participate in any EQAS that is in operation.

➢ Requirements of IQC

- Comprehensive: Cover all steps from collection of sample to reporting.
- Regular continuous monitoring.
- Rational: Focus more on critical factors.
- Practical: Should not attempt to evaluate everything.
- Economical: Should be cost-effective and within the provided budget.

Each laboratory should have Standard Operating Procedure Manuals (SOPMs) which should include the following information about the infrastructure of a laboratory

- Biosafety precautions
- Disposal of infectious waste
- Collection, transport and storage of specimens
- Criteria of rejection of samples
- Processing of specimens
- Maintenance of equipment
- Recording of results
- Reporting of results
- Procedure of quality control
- Referral

SOPMs should be periodically reviewed and revised and religiously followed in the laboratories.

Most of the above-mentioned factors have been described in various chapters of this document. The remaining has been presented briefly here.

Maintenance of equipment

Good quality equipment is absolutely essential to generate quality results. Care of the equipment purchased is also crucial.

The quality control steps for some of the commonly-used equipment at the intermediate/peripheral laboratory level is depicted in Table.

Table: Suggested maintenance of commonly-used equipment

Equipment	Maintenance Instructions
Autoclave	Clean and change water monthly Adjust water level before each run Record time, temperature and pressure for each run Inspect gasket in the lid weekly Technical inspection every six months
Incubator	Clean inside walls once in a month Record temperature at the start of each working day Technical maintenance every six months
Hot air oven	Clean the inside at least once a month Record time and temperature with every run Technical inspection every six months

Microscope	Wipe lenses with lens paper at the end of each day's work Protect the microscope from dust, vibrations and moisture Place a shallow plate containing dry blue silica gel in a box to absorb moisture Check alignment of the condenser once a month Technical inspection once in a year
Balance	Keep the balance and weights clean and dry Always use a container or weighing paper, do not put material directly on the pan Prevent the balance from drafts of air
Refrigerator	Place at least 10 inches away from the wall Clean and defrost at least every two months Record temperature daily Technical service at least once a year
Water Bath	Check water level daily Check temperature before and during use Clean monthly Technical inspection once in six months
Inspissator	Check temperature daily Clean after each batch of culture media prepared
Centrifuge	Wipe inner walls with antiseptic solution weekly Check brushes and bearings every six months

Glassware	Discard chipped glassware Ensure these are free of detergents Do not store sterile glassware for more than three weeks before it is used.

Preparation for laboratory tests

What you need to know before performing laboratory tests?

Laboratory testing is a medical procedure to verify that the blood, urine and other body samples of the materials comply with the rules and / or characteristics.

Laboratory tests are carried out:

- screening time to assess the potential human health effects even before the appearance of symptoms of disease
- diagnosis, treatment plan, assess response to therapy prescribed and follow the progress of the disease.

Laboratory test result values are usually presented as a certain range, less than a specific number, because normal values vary for different individuals.

What is the rate for one person may not be to another rate. Many factors (eg .: gender, age, race, medical history, general health) can affect test results. Sometimes the results determined by specific foods, medicines, before the start of the investigation, and whether they have been used correctly. That's why the patient may be asked not to eat or drink for several hours before the test. In addition, the rate may be different rates in different laboratories, as studies carried out at different analyzers and with different methodologies.

Some laboratory tests (eg., Prostate specific antigen, cholesterol) accurately and reliably indicate the specific health problems. Others provide general information to enable the doctor to judge the potential health problems contribute to the selection of other investigations diagnosis.

Before carrying out instrumental research experience how to prepare them properly.

Preparation for blood tests:

General requirements

1. Before the blood test 8-14 hours. (depending on the type of research carried out) Do not eat, drink, juice, tea, coffee, especially with sugar. Drink only water.
2. At 1-2 days before the test, eat less fat and fried foods, do not use alcohol. If a laboratory tests performance on the eve of planned holiday, better put off the inquiry 1-2 days.
3. Hour before the study advised not to smoke.
4. Many enzymes and hormones in the blood at various times of the day can vary, so these studies do up to 10 hours in the morning.
5. Before the blood test to avoid being in the sun, exercise and stress. Before the examination, calm down and relax for 10-15 minutes.
6. If you are going to start taking medication, then better does the research before you start to use them, or 10-14 days after treatment with medication. Determine the efficacy of the treatment is usually studied in blood after 14-21 days after dosing. If you are taking medication, be sure to warn them about your doctor or laboratory personnel.
7. Women's hormonal research outcomes are affected by physiological factors associated with the menstrual cycle. Therefore, in preparation for the study of sex hormones, specify the cycle phase and follow your doctor's recommendations, the day of the cycle should be performed.
8. Before the study of stress hormones (ACTH, cortisol, prolactin, etc.). Need to calm down during the procedure to ignore extraneous factors, relax, because it increases the stress hormones in the blood.
9. When testing for infection should be considered in the development stage of infection and immune status. A negative test results, still no guarantee that there is no infection.
10. If the results of laboratory tests make you doubt, it is 3-5 days after re-testing. The best research on infectious diseases carried out within 21 days after the suspected infection, the antibody production is active.

Venous blood obtained by venipuncture:

1. Label collection tubes and pre-cleaned slides (preferably end-frosted) with the patient’s name (or other identifier) and date (also time, preferably) of collection.
2. Clean the site well with alcohol; allow to dry.

3. Collect the venous blood in a vacuum tube containing anticoagulant (preferably EDTA); alternately, collect the blood in a syringe and transfer it to a tube with anticoagulant; mix well.
4. Prepare at least 2 thick smears and 2 thin smears as soon as possible after collection.

Memo to the patient for urine collection

- For all urine tests the best is the morning urine.
- The overall urine advised 24 hours do not use vitamin C preparations.
- Before collecting urine tests by PCR, do not pee 3-4 hours. And do not wash external genitals.

Urinary bladder assembly overall investigation and for crop:

1. Before collecting the urine you should wash genital organs (water only), women do not need to dry yourself before urinate perineum.
2. The study collected 100-200 ml of clean urine middle portion in a special jar (can be bought in pharmacies), urine culture - only in a sterile jar (sealed packaging).
3. Cup inscribed on the patient's name.
4. Urine sample must be no later than 1-2 hours delivered to the laboratory (if delivered from further ensure that the delivered +2 - + 8 ° C).

Daily urinary collection:

1. Daily urine collection procedure best done when you are at home all day.
2. Make one or more containers to collect and store urine.
3. Brown-colored plastic container is suitable.
4. Urine collection container and store in dark place (on ice or in a refrigerator) the next 24 hours.
5. First thing in the morning urine portion disposed in the toilet, it will be a reference at the daily collection of urine.
6. 24 hours after the start time, end of the urine collecting process of the urine collecting container / containers.
7. If the doctor had not informed otherwise marked collected daily urine amount, mix all available urine, separate 200 ml of urine into another container and put the record of the patient's name, and return it to the lab, ensuring +2 - + 8 ° C.
8. The definitive diagnosis of urinary schistosomiasis *(Schistosoma haematobium)* is established by demonstration of *S. haematobium* eggs in

urine. An increased number of eggs are shed in the urine around midday, so an optimum urine specimen for diagnosis should be collected at noon. The specimen should be immediately centrifuged at 400 × *g* and the sediment examined by wet mount.

9. *Trichomonas vaginalis* motile trophozoites may also be found in the urine, especially in infected male patients. To look for the presence of trophozoites, the urine specimen should be centrifuged at 400 × *g*, the sediment mixed with a drop or two of saline, and examined by wet mount. Temporary stains, such as methylene blue or malachite green, are also helpful.

Urine collection of sexually transmitted pathogens detection by PCR:

1. "Primary portions 'urine is the most appropriate for investigation.
2. Is collected in a sterile bottle 50-100 ml of urine, starting from the first drop.
3. On the cup should be written the patient's name.
4. The sample must be stored in the refrigerator and up to delivery to the laboratory is considered +2 - + 8 ° C.
5. Urine sample must be delivered within 24 hours to the laboratory.

Urine collection to identify Mycobacterium tuberculosis by PCR:

1. The study collected 100-200 ml of clean urine middle portion in a special jar.
2. Cup inscribed on the patient's name.
3. Urine sample must be at least 2-4 hours delivered to the laboratory (room temperature).
4. A special container for urine collection can be purchased at pharmacies.

Fecal sampling:

1. Stool samples should be taken from several different places, and shall not contain impurities of urine.
2. Testing is usually enough small amount of stool (about 10 g.).
3. Stool tests are not suitable feces after treatment enemas, suppositories, laxative and other medicines that affect the formation of fecal consumption.
4. The total fecal examination special training is required.
5. If the test is done on worms testing, sampling remains the same, but divorced in stool worms, it must be delivered to the laboratory along with a

stool sample.

Fecal collection for survey:

1. Feces shall be taken in a special jar (container) or washed glass or plastic bowl.
2. Cup inscribed on the patient's name.
3. Container immediately be placed in the refrigerator and up to delivery to the laboratory is considered +2 - + 8 ° C.
4. Fecal material must be delivered within 24 hours to the laboratory.

Fecal collection for protozoan cysts investigation:

1. Feces shall be taken in a special jar (container) or washed glass or plastic bowl.
2. Cup inscribed on the patient's name.
3. Stool sample (at room temperature) must be no later than 1-2 hours delivered to the laboratory.
4. Special container stool collection can be purchased in pharmacies.

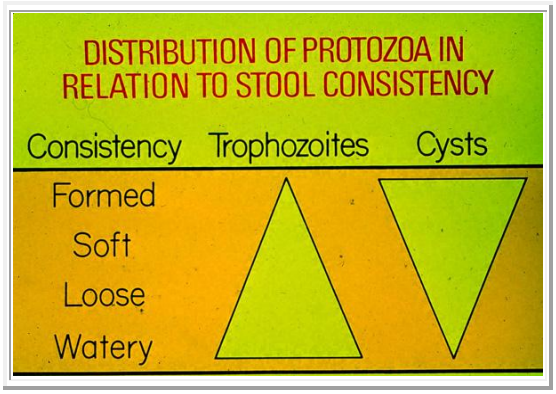
Fecal sampling sensitive detection of intestinal bleeding:

1. It is important that samples wouldn’t be taken at the time of menstruation and bleeding from haemorrhoids nodes.
2. Using fecal collectors is recommended in order to prevent urine, blood, and toilet water impurities.
3. Anticoagulants such aspirin or coumarin, can lead to higher hemoglobin fecal samples.
4. Large doses of iron products should be discontinued one or two days before taking the test sample.
5. Keep a tube at room temperature before using.
6. Take a stool sample using a stool sample collector.
7. Keeping the sample collector upright, open the screw cap.
8. Remove the spiral wand. Insert the helical rod in three different stool sample locations.
9. Place the spiral stick together with a stool sample to the sample collector and close.
10. Mark the sample collector tightly closed.
11. Keep the sample collector during the night in a cool, dark place.
12. Buffer in stool samples must be delivered to the laboratory within 5 days.

Laboratory investigation of Parasitic Diseases

Specimen Collection

1. Collect the stool in a dry, leak proof container uncontaminated by urine, water, soil or other extraneous material.
2. This table demonstrates the distribution of protozoa in relation to stool consistency and should be taken into consideration when specimens are received.



3. Fresh stool should be examined, processed, or preserved immediately. An exception would be specimens kept under refrigeration when preservatives are not available; these would be suitable for antigen testing only.
4. Preserve the specimen as soon as possible. If using a commercial collection kit, follow the kit's instructions. If kits are not available, the specimen should be divided and stored in two different preservatives, 10% formalin and PVA (polyvinyl-alcohol), using suitable containers. Add one volume of the stool specimen to three volumes of the preservative.
5. Insure that the specimen is mixed well with the preservative. (Formed stool needs to be well broken up.)
6. Insure that the specimen containers are well sealed. Reinforce with parafilm or other suitable material. Insert the container in a plastic bag.
7. Certain drugs and compounds will render the stool specimens unsatisfactory for examination. The specimens should be collected before these substances are administered, or collection must be delayed until after the effects have passed. Such substances include:

antacids, kaolin, mineral oil and other oily materials, non-absorbable anti-diarrheal preparations, barium or bismuth (7-10 days needed for clearance of effects), antimicrobial agents (2-3 weeks), and gallbladder dyes (3 weeks).

8. Specimen collection may need to be repeated if the first examination is negative. If possible, three specimens passed at intervals of 2-3 days should be examined.

Stool Examination

Macroscopic examination:

for physical characters:

Color, consistency, odor, pH & presence of blood, mucus & worms (adults or segments).

Stool specimens can be examined fresh or preserved.

Examination of fresh specimens permits the observation of motile trophozoites, but this must be carried out without delay. Liquid (diarrheic) specimens (which are more likely to contain trophozoites) should be examined within 30 minutes of passage (not within 30 minutes of arrival in the laboratory!), and soft specimens (which may contain both trophozoites and cysts) should be examined within one hour of passage. If these delays cannot be respected, the specimens should be preserved to avoid disintegration of the trophozoites. Formed specimens (less likely to contain trophozoites) can be kept for up to one day (with overnight refrigeration if needed) prior to examination.

Microscopic examination:

1- Saline & Iodine Wet preparations

Saline Wet preparations:

Method: place a small drop of *0.85%* saline on a glass slide, mixing with a very small portion of stool using a wood applicator then coverslip is placed

Value: detect eggs and larvae of helminths and motile protozoan trophozoites.

Iodine Wet preparations:
Method: A drop of Lugol's iodine mixed with a small portion of stool on slide and cover-slipped
Value: detect protozoan cysts

Preservation of specimens is necessary when the stool specimens cannot be examined within the prescribed time interval (see above). Various preservatives are available, with the two most commonly used being 10% aqueous formalin and PVA (polyvinyl-alcohol).

Because 10% formalin and PVA have complementary advantages, it is recommended that the specimen be divided and preserved in both types of preservatives (add one volume of stool to three volumes of the preservative. Preserved specimens can be stored for several months.

Specimens preserved in formalin can be tested directly (wet mount, immunoassay, chromotrope stain) or can be submitted to a concentration procedure prior to further testing.

2- **Concentration procedures** separate parasites from fecal debris and increase the chances of detecting parasitic organisms when these are in small numbers. They are divided into flotation techniques and sedimentation techniques.
Flotation techniques (most frequently used: zinc sulfate or Sheather's sugar) use solutions which have higher specific gravity than the organisms to be floated so that the organisms rise to the top and the debris sink to the bottom. Their main advantage consists in yielding for examination a cleaner material than the sedimentation techniques. The disadvantages of most flotation techniques are that the walls of eggs and cysts will often collapse, hindering identification; and that some parasite eggs do not float.
Sedimentation techniques use solutions of lower specific gravity than the parasitic organisms, thus concentrating the latter in the sediment. Sedimentation techniques are recommended for general diagnostic laboratories because they are easier to perform and less prone to technical errors.

1. **Formalin-Ethyl Acetate Sedimentation Concentration**

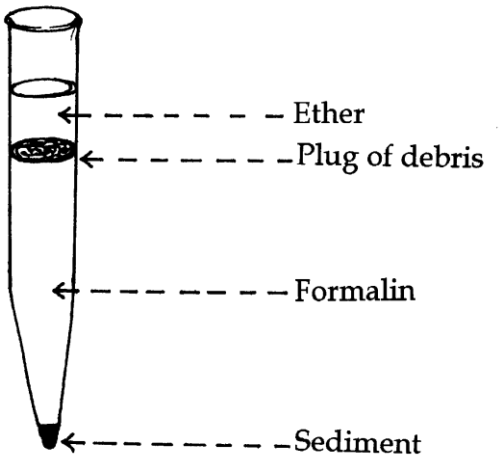
Value: detect helminthic eggs and larvae, protozoal cysts and to a lesser extent

trophozoites
Method:
-In centrifuge tube, emulsify 1g feces in 15 ml saline, strain through two layers of gauze then centrifuge (1 minute at 2000 rpm)
-Add to sediment 10 ml of 10% formalin for 10 mm then 3 ml ethyl acetate
-stopper the tube, shake vigorously in inverted position for a minute & centrifuge (1 minute at 2000 rpm)
Four layers formed: ethyl acetate, fecal debris, formalin & sediment at the bottom
-Loosen the plug of debris with applicator stick, decant everything except sediment
-mix few drops of formal with sediment and examine as wet preparation

Specimens preserved in PVA are mostly used for permanent staining with trichrome. Prior to staining, they are processed as follows:

1. Insure that the specimen is well mixed.
2. Prepare a smear using 2 to 3 drops of the specimen depending on density.
3. Heat fix on slide warmer set at 60^{o}C for 5 minutes or air dry completely at room temperature.

Slides may be trichrome stained or kept for several months in a slide tray or box for protection for future staining.



2. Zinc sulfate floatation procedure:

Value: for eggs, larvae & cysts but unsuitable for *Schistosoma mansoni* and operculated eggs
Method:
-In centrifuge tube, emulsify part of feces in saline, strain through two layers of gauze then centrifuge
-Decante supernatent & replace it by 33% zinc-sulfate solution, mix well & centrifuge
-Using a wire loop, examine the top fluid for eggs, larvae & cysts

3. Sheather's sugar floatation procedure:

Value: for *Cryptosporidium* oocysts and *Isospora* oocysts
Method:
-In centrifuge tube, emulsify part of feces in Sheather's sugar solution, then centrifuge (5-10mm at 350xg)
-using a wire loop, make a plain wet preparation (Do not add saline or iodine)
-Examine under phase-contrast microscopy. Oocysts appear as refractile structures.
-Stained by acid-fast stains

(3) Recovery of Nematode larvae from feces or soil
Larvae of hook worms, *Strongyloides and Trichosfrongylus* are recovered by:

1-Stool Culture
<u>I-Charcoal Culture</u>:
-In a petri dish emulsify a piece of stool with water & animal charcoal (prevent putrefaction)
-covered by another petri dish containing wet filter paper (provide moisture) & kept in worm place for 1 week
-Larvae, if present will migrate to wet filter paper

<u>lI-Test tube culture</u>:

-Thin film of feces spread on middle of filter paper strip & put in a conical centrifuge tube containing some water at its bottom touching lower end of filter paper
-Leave in warm place for 1 week. Capillary flow of water upwards through paper keeps feces moist
-Larvae migrate to water to be seen & examined

2-Baermann's technique: depends on +ve hygrotropism & thermotropism of larvae
-Portion of feces or soil placed in piece of gauze over wire sieve in a glass funnel (filled with warm water till lower surface of sample) {In hot weather put a piece of ice on top of the sample} having rubber tube with clamp
- Larvae (if present) migrate to water after 30 min.
-Release the clamp & examine water after centrifugation for larvae

(4) Egg counting techniques

Kato thick smear technique:
Put 50 mg feces on clean slide, covered with a strip of cellophane paper {which soaked for 24 hours in 100 ml pure glycerin, 100 ml water & I ml 3% malachite green}
Slide inverted & pressed against an absorbent surface till fecal mass covers an area of 20-25 mm in diameter
Leave for 1 hour (allow clearing of fecal material) need a lunger period of clearing (For 24 hours if detecting *Schistosoma mansoni* eggs)
The number of eggs in 50 mg feces counted

(5) Permanent stains: confirm morphological details of protozoa
-Thinly prepared slide of see-through thickness from a PVA-preserved sample is the sample of choice & the slide is air-dry.
-3 commonly used permanent stains are: •Trichrome stain •Iron haematoxylin stain •Modified acid-fast stain
**Modified acid-fast stain for
-oocysts of *Cryptosporidium, Isospora* and *Cyclospora* (oocysts absorb red color of carbol fuchain)
-differentiation between *T. solium* and *T. saginata* eggs

Urine examination
specimen collected in a clean container, centrifuged immediately

Microscopic examination of sediment reveal:
-*S. haematobiurn* eggs
-*Trichomonas vaginalis* trophozoites
-*Enterobius* eggs in urine of females
-*Wuchereria bancrofit* microfilaria in chyluria

Sputum examination

Microscopic examination of sputum is used in identifying *Paragonimus westermani* eggs, *Strongyloides stercoralis* larvae, *Ascaris lumbricoides* larvae, hookworm larvae, and *Entamoeba gingivalis and rarely E. histolytica* (pulmonary amoebic abscess eroding a bronchus)
A sputum sample can be examined in several ways:

- The unfixed specimen may be centrifuged and then the sediment examined as a direct wet mount.
- If the sputum is too viscous, an equal volume of 3% sodium hydroxide may be added, then centrifuge, and examine the sediment.

Blood examination

In suspected malaria and babesiosis, blood smears should be obtained and examined without delay.

Since the parasitemia may fluctuate in many species (the most frequently cited example being malaria), multiple smears might be needed. These can take at 8 to 12 hour intervals for 2 to 3 days.

Microfilaremias exhibit a marked periodicity depending on the species involved, and the timing of specimen collection may be critical. If infection is suspected, the optimal collection time is as follows:

Microfilariae:
Loa loa–midday (10 AM to 2 PM)
Brugia or Wuchereria–at night, after 8 PM
Mansonella–any time

Types of Sample:
Venous blood samples provide sufficient material for performing a variety of diagnostic tests, including concentration procedures (filariasis, trypanosomiasis).

However, in some infections (malaria in particular), anticoagulants in the venous blood specimen can interfere with parasite morphology and staining characteristics; this problem can be further compounded by excessive delays prior to making the smears. In such cases, capillary blood samples are preferable.

Capillary blood obtained by fingerstick:

1. Label pre-cleaned slides (preferably end-frosted) with the patient's name (or other identifier) and date (also time, preferably) of collection.
2. Clean the site well with alcohol; allow drying.
3. Prick the side of the pulp of the 3rd or 4th finger (alternate sites include ear lobe, or in infants large toe or heel).
4. Wipe away the first drop of blood with clean gauze.
5. Prepare at least 2 thick smears and 2 thin smears.

Specimen Processing

Thick smears: for screening parasites particularly malaria
Method -Put few drops of blood (3 or 4) on a slide
-Spread blood by comer of another slide (to form a uniform smear)
-Let the slide air-dry, Immerse in distilled water (to haemolyse RBCs)

Thin smears: for identification of species
Method -Place one drop of blood close to one end of the slide
-Hold another slide at an angle of 45^0 to the first one and let it touches the blood drop. After blood spreads along its edge, move it steadily along the length of the slide

Staining procedure:
Giemsa stain: preferred
The thick or thin film is covered with freshly diluted stain in buffered distilled water (1: 20) for 20 min., wash under running water & leave to dry

Knott Technique:
Concentrates microfilariae. Blood (1-5cc) mixed with 2% formaline & centrifuged
Smears of sediment air-dried, Giemsa stained & examined.

Buffy Coat smears:

Done after a routine haematocrit. by collecting oxalated blood, placing it in a wintrobe tube and spinning it for 30 mm at 100 x g. 3 layers formed: packed RBCs at the bottom, buffy coat then plasma.

Buffy coat taken by a capillary pipette from Wintrobe tube & placed on a glass slide with a small amount of plasma

Stained with Giemsa & examined microscopically for *Leishmania* and *Trypanosoma*.